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Effect of some nutritional supplement on the cerebellum against cyclophosphamide toxicity

Ali Hassan A Ali[1,2]✉, **Shaban Ragab Ibrahim**[3,4], **Abdulrahman M Alkassar Alanazi**[5], **Hamad Mesfer H Alatif**[5], **Bandar Suliman S Alsultan**[5], **Bakheet Mulfi S Alrashdi**[5], **Yousef K Alhuzaimi**[5], **Obaid A M Alhajri**[5], **Abdulhakim Alqahtani**[5]

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Author Affiliation:
[1]Anatomy Department, College of Medicine, Prince Sattam Bin Abdulaziz University, Alkharj 11942, KSA
[2]Anatomy Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[3]Forensic Medicine and Toxicology Department, Prince Sattam Bin Abdelaziz University, Saudi Arabia Alkharj 11942, KSA
[4]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
[5]College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA

✉**Corresponding author**
Anatomy Department, College of Medicine, Prince Sattam Bin Abdulaziz University, Alkharj 11942, KSA
Anatomy Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt
Email: alihassan3750@yahoo.com & a.ali@psau.edu.sa

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ABSTRACT

The cerebellum is anatomically situated behind the brain stem inside the posterior cranial fossa. As an important part of nervous system, it can be affected by oxidative stress. Dietary supplements have been shown to have antioxidants properties that may protect our bodies from the dangerous effects of free radicals. One such supplement is Aphanizomenon flos-aquae (AFA) which has health-improving effects particularly on the nervous system. Cyclophosphamide (CP) is a widely used chemotherapy drug that can cause an oxidative stress. This study was carried out to investigate the role of AFA in prohibiting the adverse effects induced by cyclophosphamide on the cerebellum of the animals treated with CP. It was carried out on 36 rats with body weights of 270-330 g. The animals were classified into three groups. The first one is control group, the second is CP treated group, received one dose of CP at 100 mg/kg, and last group is CP+ AFA group, received orally extract of AFA after CP injection. The structure of the cerebellar tissue was compared in the different groups histologically. The examined sections showed significant cellular injury in the second group compared to the control group. The third group treated with AFA after CP injection showed marked improvement in the changes that occurred compared to the second group. These results provide evidence that AFA has a protective effect as it reduces the cellular injuries in the cerebellum induced by cyclophosphamide.

Keywords: Cerebellum, Antioxidant, Cyclophosphamide, Rat, AFA, Food Supplement.

1. INTRODUCTION

The cerebellum is in charge of coordinating skilled voluntary movements. Also, it is active precision and kinesthetic learning, as well as in timing of movements. It is different from other parts of the brain because it goes

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through the time of its prime development from the 3rd trimester to infant period (Salem et al., 2010). As a result, the cerebellum is highly liable to damage especially in young children (Wang et al., 2014). The cerebellar cells have a generate capacity, and this depends on the consumption of food and therefore the concentration of amino acids in the bloodstream (Gouda et al., 2010; Keshavarz et al., 2013). The cerebellum is important for making adjustments in pasture in order to maintain balance. One of the main functions of the cerebellum is to coordinate the timing and strength of these different muscle groups to produce body movements (Drake et al., 2020).

AFA (Aphanizomenon flos-aquae) is an algal species gathered every summer in Oregon area one of the United States. It was sold as a food supplement for about 20 years as its nutritional benefits of AFA have been liked by many people (Bruno, 2001). Aphanizomenon flos-aquae (AFA) directly metabolize molecular nitrogenin the air and form many Low Molecular Weight Peptide Groups. These peptides are neurotransmitters that are used across different brain areas and body to release other substances and influence metabolic functions. It has been confirmed that AFA is a good origin of Omega-3 and 6 that build nerve fibers in the brain (Cunnane et al., 2009).

Cyclophosphamide (CP) is one of the common chemotherapeutic drugs that ceases the malignant cell growth and inhibits the immune system. It has a good application in a variety of malignant and non-malignant tumors (Shanafelt et al., 2007). It is used for the treatment of many types of cancers, multiple sclerosis, and other benign tumors. Also, it is used in the treatment of nephrotic syndrome and following an organ transplant (Singh et al., 2019).

Our aim is to study the protective effect of AFA on the cerebellum of albino rats against the cyclophosphamide-induced hazards effects.

2. MATERIALS AND METHODS

CP was obtained from Germany (Baxter Oncology-Frankfurt). Klamath AFA- capsules (350 mg) have been bought from Egypt (GEPC-German Egyptian Pharmaceutical Company) were melt in distilled water. It was administrated orally via a gastric tube. The dose was 94.5 mg/kg /BW/day for 30 days. This study is an experimental study carried out in the period from January 2021 to August 2021.

In our research, 36 healthy, aged 9 weeks, male albino rats (270-330 g.) were used. They were obtained from an animal house at PSA University. At the Animal Care Facility, they were kept under standard animal housing conditions. The rats were stayed under supervision for about 2 weeks before the start of the study for acclimation. The animals were differentiated into three groups of twelve; the first one was considered as a control group. The second group received intraperitoneally one dose of CP (100 mg/kg BW). Before beginning our study 100 milligrams of CP injected into four rats to ensure significant histological changes in the cerebellum. The dose was selected on the basis of previous studies. The latter group received AFA extract orally 500 mg/kg per day for 30 days after intraperitoneal cyclophosphamide injection.

For histological studies; after a month, the rats were sacrificed and after dissection, small pieces from the cerebellum were taken. Specimens were prepared for fixation in formalin. Then, sections stained with Harris haematoxylin and eosin (Hx&E). A few sections stained to find out polysaccharides. For detection of Nissel granules, we used Toluidine blue stain. Apoptotic changes and programmed cell death was detected in the cerebellum of all groups by Caspase-9 immunostaining.

The image J 1.4 analyzer was used to take out the morphometric data. For example, carbohydrate content of the cerebellar cells using PAS-stained and Caspase-9 immnuostained sections were used. PAST 3.0 Version of statistical analyses was done. The obtained data were expressed as mean ± standard deviation (SD). The $p<0.05$ was significant.

3. RESULTS

The H&E stain examination of the control group: the cerebellum showed its three layers the molecular layer, the Purkinje cell layer, and the granular layer (Fig. 1, 2). In the cerebellar white matter, few neurons were found with their nerve fibers. Periodic Acid Chief (PAS) showed well organized PAS response on normal neurons. Toluidine blue stain showed a strong blue stain of Nissel granules in the cerebellar Purkinje cells. In addition, immunohistochemical studies of brain sections showed a mild expression of Caspase-9 immunostaining for neuronal cell bodies and glial cells.

The second group took cyclophosphamide (CP) stained with Hx. & E showed disfigured Purkinje cells of various shapes. Some Purkinje cells appeared either degenerated or with karyolytic nuclei. Other cells appeared with shrinkage of their cytoplasm and nucleus pyknosis. The PAS reactions of the cerebellar cells of this group showed mild and moderate reactions to PAS in degenerated neurons. Weak reactions to Nissel granules; shown Toluidine-blue staining sections of the cerebellar cells. A

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significantly increased Caspase-9 expression in deteriorating neuronal cell bodies with Immunohistochemical examinations when compared to the control groups (Figs. 3 and Table 1).

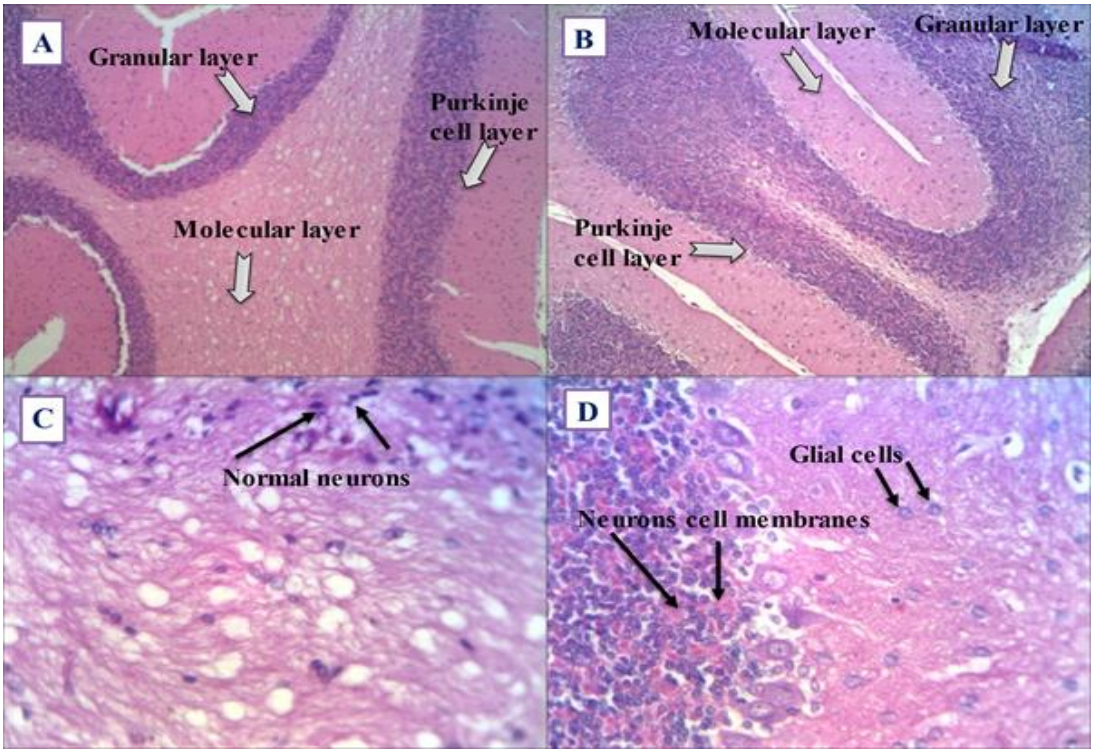

Figure 1 (A, B) Hx. &E. of the cerebellum of control group showing all layers (Purkinje cell, granular and molecular) (X200). B, C) The control group showing a powerful reaction to PAS +ve in the neurons of the cerebellum and also in the glial cells (X400)

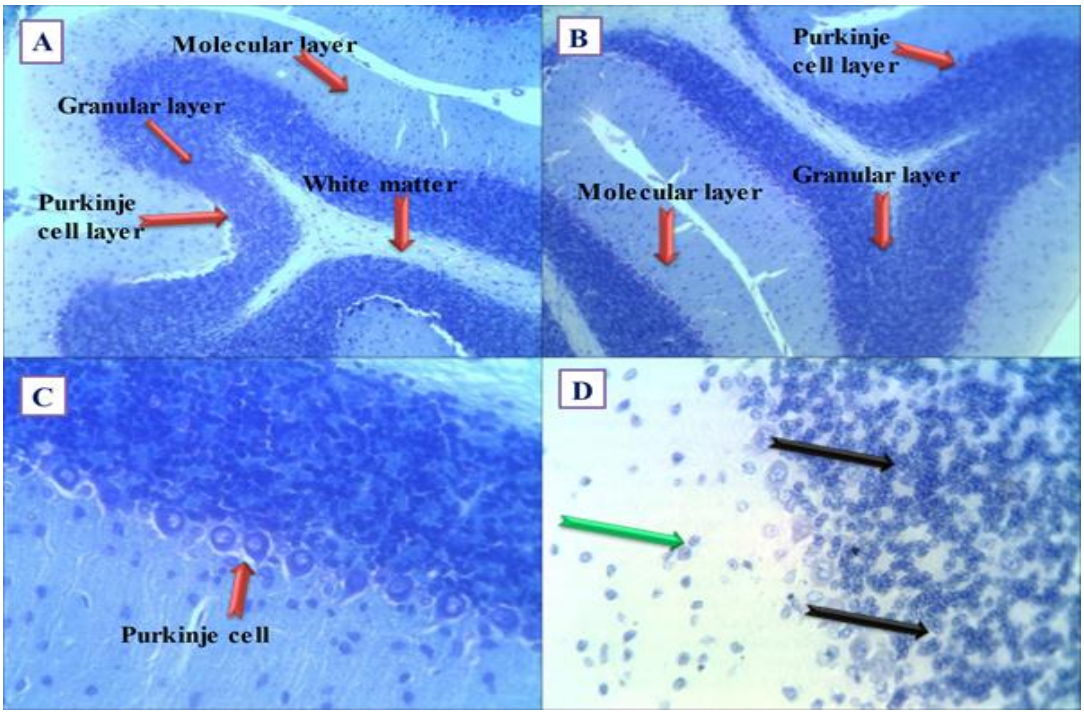

Figure 2 (A, B) Toluidine blue stain of the cerebellum of the first (control) group showing all layers (Purkinje cell, granular and molecular) (X200). C) Toluidine Blue stains of cerebellum offirst (control) group showing high cellular content of the Purkinje cells Nissel granules. Cells show dark blue cytoplasm as well as flask like shape (X400). D) Caspase 9 immunostaining in neuronal cell bodies of the cerebellum showing normal neuronal cell bodies (black arrows) and also in the in glial cells (green arrow) (X400).

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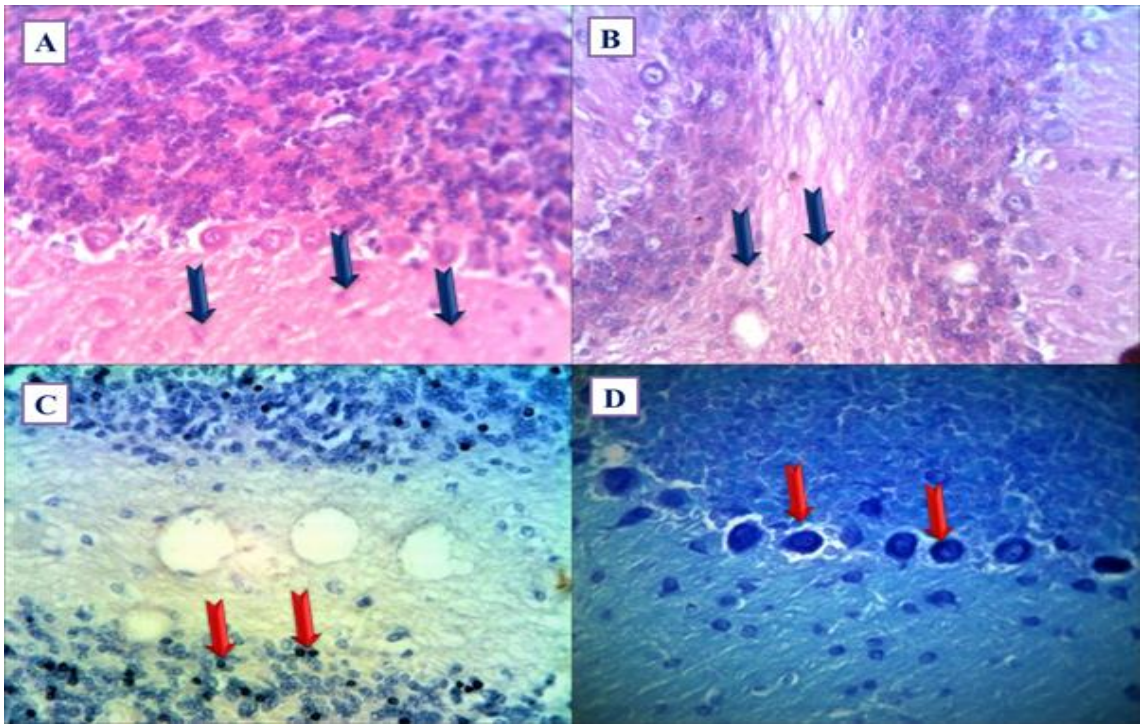

Figure 3 (A, B) PAS reaction of some cerebellar cells (blue arrows) and a mild PAS reaction in deteriorated neurons of the medulla treated with cyclophosphamide (CP) (PAS, X400). C) Toluidine Blue stain of the treated group with CP exhibiting a decrease in cellular content of Nissel granules in Purkinje cells as well as degenerated cells (red arrows). D) Caspase-9 immunostaining in degenerating (red arrows) cerebellar cell bodies (X400).

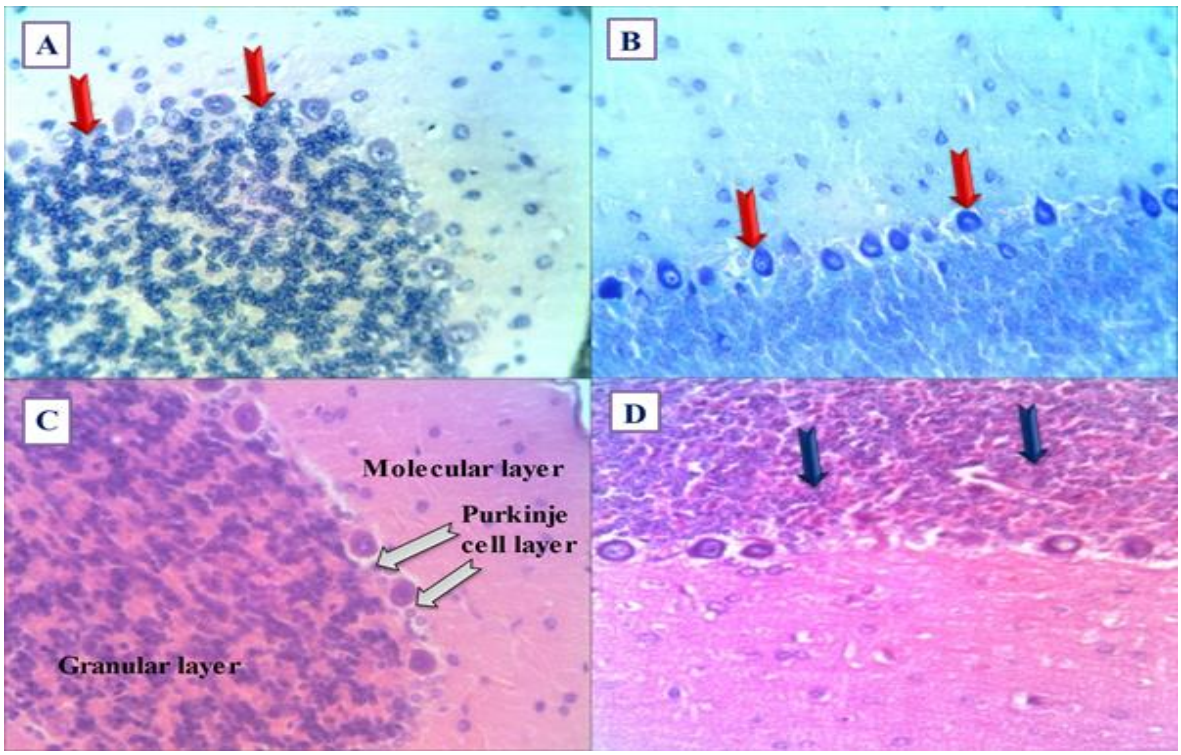

Figure 4 Cerebellar tissue of group treated with AFA showing A) Mild expression of Caspase-9 immunostaining in the cells of the cerebellum (red arrows) B) Strong basophilic stained cytoplasm of Nissel granules in Purkinje cells (red arrows) with Toluidine blue stain (X400). C) Hx. & E of the cerebellum of group treated with AFA almost near to control group cells. D) Strong reaction to PAS staining in almost normal neurons as in the first group (blue arrows) (X400).

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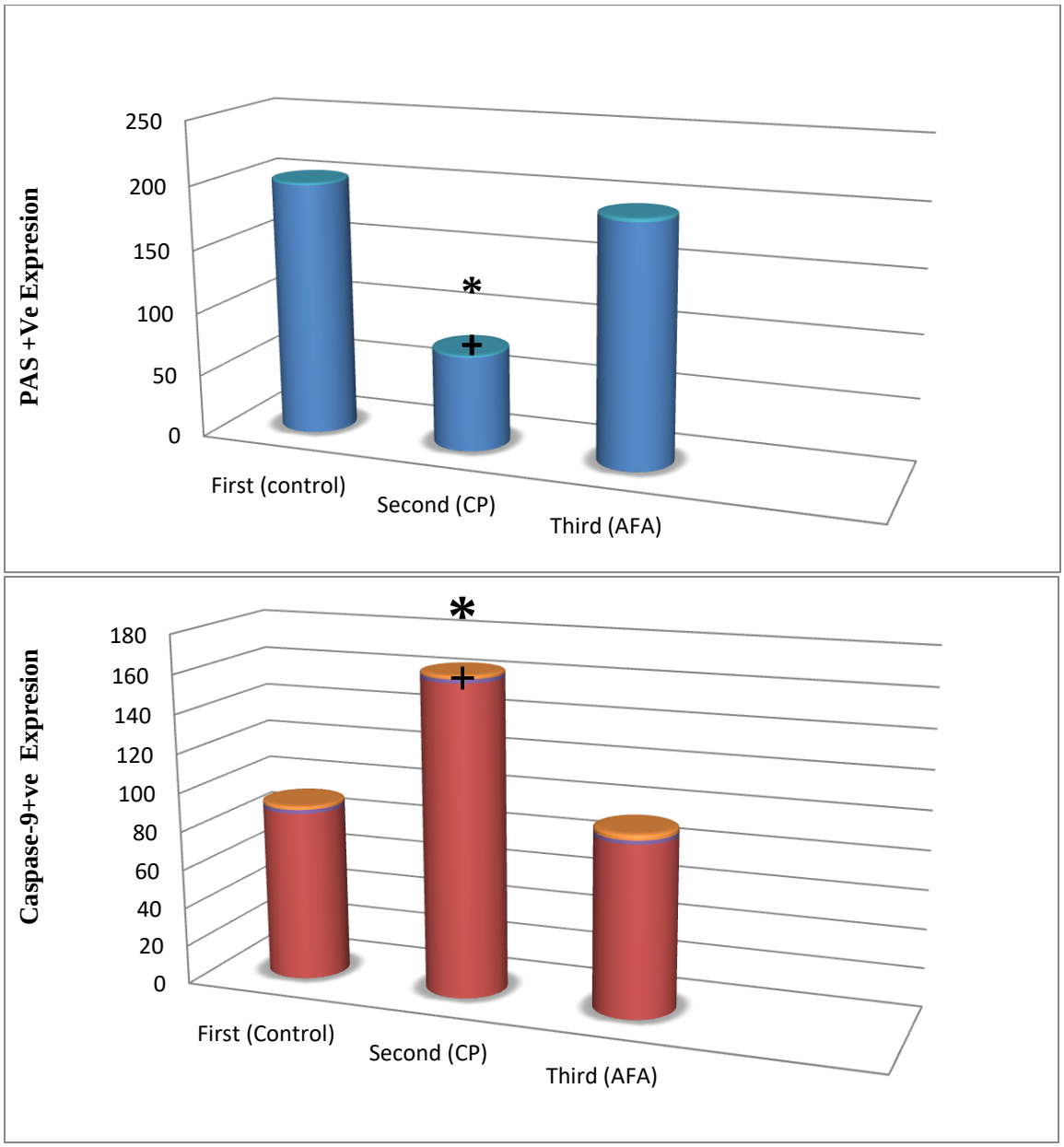

Figure 5 PAS^{+ve} and Caspase-9^{+ve} expression of Optical Density expression of the cerebellum for all studied groups.

The third group was treated with AFA extract stained with Hx. & E showed an effective preventing of degenerative changes in most of cerebellar cells. They appeared almost similar to the first group. The Purkinje cell layer as well as cerebellar medulla appeared nearly similar to the first (control) group. When the same group stained with Toluidine blue; showed marked increase in Toluidine blue stain with the intensity of Purkinje cells. With Periodic Acid Schiff stain showed marked and strong reaction. In addition, this group when treated with Caspase-9 immunostaining in the distorted cerebellar cells exhibited a marked increase in the expression of Caspase-9 (Fig. 4, 5 and Table 1).

Table 1 PAS^{+ve} and Caspase-9^{+ve} of the Cerebellar cells for all studied groups-mean ± SD.

Studied Group Parameters	PAS+ve Cerebellar cells	Caspase-9+ve Cerebellar cells
Control group	199.10± 43.62	87.6± 11.15
CP group	75.30± 14.03*	159.6± 26.67*
AFA group	189.50± 24.51	88.00± 27.48

*Significant means a rise or decrease in the level's parameter (P<0.05).

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4. DISCUSSION

Cerebellar cells are particularly at risk of destruction by induced free radicals due to high amount of iron. Besides; the brain has a relatively weak defense mechanism for antioxidant (Kumar et al., 2015). The purpose of our research was to assess the protective effect of AFA on the cerebellum of rats against the cyclophosphamide-induced hazards effects. Our study demonstrated that normal overall structure of the cerebellum of control rats. It was harmonic with previous study (Eltony et al., 2010). A few researches referred this effect to the rise of Reactive Oxygen Species (ROS) formation accompanied with complications of CNS like cerebrovascular complications, decreased cerebral blood flow, disturbance of blood-brain barrier and brainoedema (Leelavinothan and Munioppan 2004; Nasr et al., 2020). Because neurons need large amounts of oxygen due to their high metabolic rate, anoxia (oxygen depletion) may give a share in the deteriorating changes present in the second group treated with CP (Gold et al., 2004). Additionally, AFA can save the cerebellum through formation of peptides of low molecular weight that start secretion of other substances (like hormones) and affect metabolic functions (Amber et al., 2018).

In our study, the medulla of cerebellum of rats treated with CP showed a weak PAS reaction, referring to reduce the amount of mucopolysaccharides in their cells. Some studies proved this before the glycogen content in the whole brain was proved to decline after lithium administration (Souza et al., 2010). Brain impairment due to oxidative stress from Reactive Oxygen Species is due to the fact that it uses about one fifth of the body's total oxygen demand and is also relatively lacking in the content of antioxidant enzymes (Seo et al., 2019). Several other researches have reported that the antioxidant activity of AFA extract resulting from synergic effect of all of its diverse ingredients (Zhang et al., 2018).

5. CONCLUSION

Taking into account the result obtained from our study, toxicity was caused by Cyclophosphamide causing alterations in the normal histological structure of the cerebellum. This risk effect can be reduced by using of Aphanizomenon flos-aquae.

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Authors' Contributions

All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A. Ali, Abdulrahman M. Alkassar Alanazi and Shaban Ragab Ibrahim participated in the study design and wrote the first draft of the manuscript. Hamad Mesfer H. Alatif, Obaid A M Alhajri, and Bandar Suliman S Alsultan collected and processed the samples. Bakheet Mulfi S Alrashdi, Abdulhakim Alqahtani and Yousef K. Alhuzaimi participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

Ethics Approval

All series of steps that were implemented in this study that included animal models were in compliance with Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (PSAU-2020 ANT 4/42PI).

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This study has not received any external funding.

Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are presented in the paper.

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